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REPORT CP 246

GEL BLEED STUDY

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REPORT CP 246

GEL BLEED STUDY

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1.0 TITLE

GEL BLEED STUDY

2.0 ABSTRACT

This communication reports the results on the in vitro diffusion of low molecular weight (LMW) siloxanes (siloxane species with molecular weight less than 1,500) and platinum (Pt) from Mentor Gel-Filled Mammary Implants into porcine serum. The diffusion experiment was carried out by immersing the implant devices in porcine serum, a system closely resembling the body fluid of women.[1-2] Aliquots of the porcine serum at various time intervals covering device immersion over a 120-day period were analyzed. The LMW siloxanes were determined by a gas chromatography/mass spectrometry (GC/MS) method after they were isolated from the serum through methylene chloride extraction. The determination of Pt was accomplished through microwave-assisted acid digestion with subsequent inductively coupled plasma/mass spectrometry (ICP/MS) analysis.

In a separate assessment, the solubility of LMW siloxanes and Pt/siloxane complex in water and/or in porcine serum was determined. The solubility assessment sets the upper limit for target compound diffusion from the devices into the media of interest.

The results indicated the migration of LMW siloxanes and Pt/siloxane complex from the devices was negligible during the 120-day study. At the end of the 120 day immersion of the Smooth Round Moderate Profile device (125 cc size) in porcine serum, only 4.3 µg total LMW siloxanes (siloxane species with molecular weight less than 1,500) and 4.1 µg Pt were measured in the porcine serum. Throughout the 120 day incubation period, only background levels of LMW siloxanes were measured in porcine serum and the extractables found in the devices remained unchanged after the 120 day immersion (CP246Addendum I). The serum Pt level followed an increasing trend initially, reached its maximum at the 60-day point, and started leveling off thereafter. From the solubility assessment and with the consideration of total target migrants found in the gel filler of typical 125cc size device, a sum of 370.3 µg for the target LMW siloxane species (D3-D6, and D9) and 528.5 µg of Pt could be expected in the serum if all are released from the device. This finding suggests the diffusion of LMW siloxanes and Pt/siloxane complex is restricted by their much stronger affinity toward the silicone network (gel filler and shell material) than the physiological fluid, the human body environment.

3.0 INTRODUCTION

Typical gel-filled mammary implant devices are comprised of shell and gel filler. The migration of gel material through intact elastomeric shell has been well documented.[3-4] Among the potential migratable materials, of particular concern are the LMW siloxanes and Pt-siloxane complex, the latter being the catalyst used in these silicone materials. High molecular weight (HMW) siloxanes are generally considered safer than LMW siloxanes due to their relatively immobile nature. The purpose of the study was to investigate the potential migration of LMW siloxanes and the Pt-siloxane complex under conditions simulating in vivo conditions. The investigation included three analytical approaches:

(1) A gel migration study where Mentor Smooth Round Moderate Profile Gel Implants were immersed in porcine serum at 37 °C. The gel migration (or gel bleed) was monitored through a time-dependent study of LMW siloxanes and Pt found in aliquots of serum withdrawn covering over a 120 day span.
(2) The loss of LMW siloxanes and Pt from gel filler as determined by comparing the content of these potential migrants in gel fillers of devices that had and had not been exposed to porcine serum.
(3) The solubility determination for LMW siloxanes and Pt-catalyst in the physiological fluids. This provides the maximum amount of these target compounds which can be expected in the test fluids as the result of diffusion because the target compounds must be soluble in the receiving media for the diffusion to occur.

This report presents the results of approach (1) after device immersion for a total of 120 days and approach (3) the solubility measurements. The results from approach (2) are presented in Addendum I of this report.

The gel migration study was conducted in porcine serum (static test) at body temperature (37°C). Three 125 cc Mentor Smooth Round Moderate Profile Gel-Filled Implant devices were tested and an M/V ratio of ~1/2 was used. Aliquots (20 mL) of the porcine serum were withdrawn at 1, 2, 4.5, 10, 20, 30, 45, 60, 74, 90, and 120 days from the start of the immersion. The porcine serum bath was replenished with fresh media following each aliquot removal. Chlorhexidine was used in the bath as a preservative to control microbial growth.[5] The reason that Smooth devices instead of S---------- ---------dition to the smooth shell Siltex devices ---------- ------- ---------- - and are less prone to gel bleed.

The solubility determination was followed according to Standard ASTM Method E 1148-02.[6] Excessive amounts of representative LMW siloxanes (D3-D6, andD9) and Pt-catalyst were mixed with the media of interest (water or porcine serum) under constant stirring at room temperature. The solubility is defined as the highest concentration of the target compounds found in aliquots of test media over different time intervals prior to an equilibrium or a declining trend was established. LMW

siloxanes were known to evaporate at room temperature over time due to their low boiling point and high vapor pressure.

A methylene chloride partitioning procedure was used to extract LMW siloxanes from water or porcine serum samples collected from the bleed study and the solubility experiments. The extracts were analyzed by GC/MS for qualitative and quantitative results. For Pt determination, portion of the serum samples was digested using aqua regia under microwave conditions for a complete dissolution of solids. The digestates were then subjected to ICP/MS analysis for total Pt content. The quality control checks included detection limit, linearity of standard calibration, method reproducibility, recovery of target analytes from methylene chloride extraction and microwave-assisted digestion. Both instruments were validated to ensure performance prior to sample analysis.

4.0 SAMPLES

Samples to be analyzed were water and porcine serum aliquots collected from gel migration study and solubility determination. All information regarding device information/ traceability and conditions for both studies are listed in Appendix B.

5.0 EXPERIMENTAL

The detailed description of experimental conditions can be found in Protocol CP246 (Appendix A).

5.1 Gel Bleed Study

The study was conducted using Smooth Round Moderate Profile Gel-Filled Implant devices (125cc). The study was carried out in triplicate and under sterile conditions. The devices were immersed in porcine serum (225 mL) containing 50 ppm chlorhexidineHCl in a sealed wide mouth glass jar at 37 °C with constant stirring. Aliquots (20 mL) were removed for analysis at 1, 2, 4.5, 10, 20, 30, 45, 60, 74, 90, and 120 days from the start of immersion. Immediately following the aliquot removal, the serum bath was replenished with freshly prepared serum containing 50 ppm chlorhexidineHCl. The glass jar was kept closed at all times. The aliquot removal and serum replenishment were accomplished through a two port design (one for aliquot withdrawal and the other for introduction of replenishing serum) on top of the lid of the jar. Each port was connected to a stopcock that facilitated the opening and closing of the port and the attachment of a 10 mL gas tight syringe for aliquot transfer (Figure 1 of Appendix A).

5.2 Solubility Determination

The determination was conducted at room temperature. ASTM Standard Test Method E 1148-02[6] was followed. For target LWM siloxanes, a mixture that contained ~5 mg each of standard D3-D6, and D9 was gently stirred (lowest setting of the stirring plate) in 400 mL water or porcine serum. For Pt/siloxane complex, only its solubility in porcine serum was determined. Pt-catalyst (~300 mg) from SiTech LLC which contained 3% Pt was stirred in 400 mL porcine serum. Aliquots (10 mL) of either water or porcine serum were taken periodically and analyzed until either equilibrium or a declining trend was observed (96 h or longer). The solution was left without stirring for 10 min prior to aliquot removal. Since siloxane compounds have a tendency to form stable small droplets upon vigorous mixing with water, all aliquots were examined under microscope to ensure the absence of the droplets prior to analysis.

5.3 Methylene Chloride Extraction and GC/MS Analysis for LMW Siloxanes

LMW siloxanes were extracted from water or serum solution through liquid/liquid partitioning with methylene chloride. Either an 8 or 10 mL water or serum solution was partitioned with 2 mL methylene chloride. A small quantity (~0.3 g) of anhydrous sodium sulfate (Na_2SO_4) was added to the methylene chloride extract to remove residual water.

For GC/MS analysis, a J&W DB5MS column (30 m x 0.25 mm I.D., film thickness: 0.25 μm) was used. The injection sample solutions were comprised of 1 mL aliquots obtained from Sections 5.1 and 5.2 and 0.1 mL internal standard solution (contained 200 ppm internal standards). The analysis was performed on 1 μL splitless injections. The injector temperature was set at 250 °C. The GC temperature program started at 20 °C (held for 3 min) and was then increased to 300 °C at 5 °C/min (held for 15 min). Helium carrier gas flow was set at 0.9 mL/min. The MS detector temperature was kept at 300 °C and it used electron impact ionization in both scan (35-550 amu) and SIM modes (most dominant ions of target analytes). Mentor 1[7] and NIST libraries were used for spectra matching routine. Retention indices data included in Mentor 1 were used for further confirmation of compound identification.

The data acquisition for quantitative analysis was conducted with the MS detection set at SIM mode. The ion selections included most predominant ions found in internal and external standards and the compounds found in samples through qualitative analysis under scan mode (35 to 550 amu). All quantitation for target analytes were based on linear regression curves consisting of multiple (at least three) standard calibrations. All standard curves had a correlation coefficient of greater than 0.95. Other compounds were quantified using the response factor of nearest internal standard.

5.4 Microwave-Assisted Acid Digestion and ICP/MS Analysis for Total Pt

Porcine serum samples (~0.5 mL) from Sections 5.1 and 5.2 were digested with 2.5 mL aqua regia and 7.5 mL water. For microwave digestion, the temperature was ramped to 180 °C in 20 min and held at 180 °C for 25 min. Under the conditions, a complete dissolution of all samples was observed.

The digestates were analyzed for Pt content following acid digest. Prior to sample analysis, ICP/MS was tuned to meet the set criteria. A typical sequence of analysis consisted of calibration blank for background check, calibration standards, and quality control standards (for verifying accuracy of calibration standards). Internal standard solution was introduced to the spray chamber from a separate reservoir at a constant rate to yield a ~10 ppb concentration in the mixed solution. The linear regression standard curve for Pt consisted of at least three standard points. The quantitation was accomplished with the use of manufacturer's built-in software.

6.0 RESULTS

All results pertaining to solubility determination are compiled in Appendix C. Appendix D contains the results from the gel migration study. All instrument validation reports are filed in laboratory log books.

6.1 Method Validation

Since both GC/MS and ICP/MS methods have been validated previously,[7-9] the validation of both methods is no longer needed. Besides instrument validation, the quality checks covered in this study included method reproducibility and method recovery for methylene chloride extraction and microwave-assisted acid digestion, detection limit, and linearity of standard calibration.

As shown, the GC/MS method provided a detection limit of <0.01 ppm for all target LMW siloxanes in porcine serum (Appendix D, Section I, Part 3). As low as 2.0 ppb Pt can be detected by the ICP/MS method (Appendix D, Section II, Part 2) in porcine serum and gel samples respectively. Satisfactory spike recoveries [ranging from 86.0 to 109.6% with one exception (D3) at 149.9%] were obtained from the methyelene chloride extraction for LMW siloxanes and the acid digestion of porcine serum with Pt-catalyst. The triplicate results were generally reproducible with a CV < 0.34.

6.2 Solubility of LMW Siloxanes and Pt-Catalyst

The solubilities of both LMW siloxanes and Pt-Catalyst in aqueous solutions (water and/or porcine serum) were in the ppm or sub-ppm ranges (Table III) due to their highly hydrophobic nature. Even though the solubility experiments were conducted in a closed glass container (same setup as the one

used for bleed study), loss of LMW siloxanes and gradual reduction in LMW siloxanes levels were observed with time. The low solubility and volatility of LMW siloxanes were believed to be the cause for the difficulty in reproducing results after multiple trials and with extended time intervals. Moreover, since porcine serum is not a clear solution, there is the presence of particles presumably protein or fat nature, which makes it even more difficult for an accurate solubility measurement. Owing to its lower volatility nature, the solubility results for Pt-catalyst were consistent. For both cases, the highest concentration of Pt and LMW siloxanes found among multiple preparations and in aliquots taken from different time intervals was chosen and reported as the solubility for the target migrants. As a consquence, the solubility of LMW siloxanes is within the same magnitude as that reported by Dow Corning.[10]

6.3 Release of LMW Siloxanes and Pt from Gel Implants into Porcine Serum

Only background or close to background levels of LMW siloxanes were released into porcine serum during the 120-day immersion (Table I). The GC/MS analysis found that the majority of the semivolatile compounds present in the serum samples were compounds associated with serum. These included aldehydes, fatty acids, esters, alcohols, and cholesterol (Appendix D, Section I, Part 4). The trace quantities of siloxanes detected in the method blank (porcine serum) or samples were D3-D6, D9, and unidentified siloxane. Among them, the identity of D9 was not confirmed due to lack of sensitivity in scan mode. Besides siloxanes, trimethylsilyltetradecanoate was the only other silicon-containing compound detected in the serum. The compound was detected after the devices had been immersed in the serum for 30 days. Only up to 0.04 ppm of the compound was measured in serum (or a total quantity of 9.0 μg from a whole 125 cc device). During the 120-day period, there seemed to be no time-dependent trend for any of the siloxane compounds. The maximum accumulated release of total siloxanes was measured 4.3 μg at the 30-day time point.

The data indicated that the release of Pt was time-dependent in the initial 40 days, the release reached a maximum (4.1 μg) at the 60-day time point and leveled off thereafter. After 120 days of incubation at 37 °C, no sign of microbial contamination was observed. The serum appeared more turbid, probably caused by protein denaturation or oxidation followed by separation of lipids from their protein carriers.

7.0 DISCUSSION

The LMW siloxane compounds (molecular weight <1500) detected in the porcine serum bath as the result of gel migration included D3-D6, D9, and an unknown siloxane. The solubility of both LMW siloxanes and Pt-catalyst is limited in serum and/or in water (in the sub-ppm and ppm range respectively). Since the solubility

determination was performed at room temperature, slightly higher values are expected for solubility at physiological temperature (37°C). The solubility data is useful; it provides the maximum allowable quantities the migrants may present in the physiological media that surrounds the implants in a fixed volume. In this study, a 125 cc size implant was immersed in 225 mL porcine serum. Based on the quantity of the migrant found in typical device gel filler and the solubility data (Columns 1 and 2 of Table IV), the value for a complete migrant release into the serum can be obtained (Column 3 of Table IV). The comparison of this value versus the bleed measurement (Column 4 of Table IV) shows only a small fraction of the available migrants were actually released into porcine serum after device immersion for 120 days. For LMW siloxanes, the total release ranged from near detection limit level for most of the analytes to 2.8 µg for D5. It was 4.1 µg for Pt. The release of Pt was time-dependent at a rate of ~0.50 ng/cm^2.day for the 125cc device during the first 40 days (Figure 1). It reached maximum at the 60-day time point. During the 120-day period, no fixed pattern was observed for the release of the LMW siloxanes and all levels found were typical background levels. Even though the bleed study was conducted in a sealed container, the loss of LMW siloxanes through evaporation is expected. This loss will be assessed by comparing LMW siloxanes content of these devices after 120 day serum immersion versus control devices that have not been exposed to serum. The results are presented in Addendum I of this report.

8.0 ACKNOWLEDGMENTS

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Table I

Release of LMW Siloxanes from Smooth Round Moderate Profile Gel-Filled Mammary Implants after Incubation in Pocine Serum (at 37°C)

Compound/Time (day)	1	2	4.5	10	20	30	45	60	75	90	120
	ug										
D3[1]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
D4[1]	NA	NA	0.469	0.042	0.042	0.042	0.042	0.042	0.042	0.042	0.042
D5[1]	NA	NA	0.581	0.052	1.720	2.844	0.435	0.435	0.435	0.435	0.435
D6[1]	NA	NA	0.675	0.060	0.060	1.391	0.178	0.178	0.178	0.178	0.178
Siloxane[2]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
D9[1]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Total Siloxanes Released per Device	NA	NA	1.725	0.153	1.822	4.277	0.655	0.655	0.655	0.655	0.655
Total Siloxanes Released per g Device (ng/g)	NA	NA	12.703	1.129	13.417	31.492	4.823	4.823	4.823	4.823	4.823
Total Siloxanes Released per Surface Area per Day (ng/cm^2.day)	NA	NA	2.540	0.102	0.604	0.945	0.096	0.072	0.058	0.048	0.036

Data presented are mean values of triplicate preparation.

ND = Not Detected, S/N <3.0

NA = Not Applicable, at least one of the replicates had a ND value.

Data preceded with a "<" symbol meaning a less than method detection limit value.

[1] Measurement based on external and internal standard calibrations.

Average Device Wt = 135.8 g

Average Surface Area of 125cc Device = 150.9 cm^2

[2] Measurement based on the response factor of closest internal standard.

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Table II

Release of Platinum from Smooth Round Moderate Profile Gel-Filled Mammary Implants after Incubation in Porcine Serum (at 37°C)

Compound/Time (day)	1	2	4.5	10	20	30	45	60	74	90	120
Total Pt Released per Device (ug)	0.410	0.544	0.924	1.223	1.835	2.350	4.004	4.058	3.505	3.705	3.243
Total Pt Released per g Device (ng/g)	3.016	4.004	6.801	9.008	13.512	17.307	29.485	29.885	25.808	27.286	23.882
Pt Released per Surface Area per Day (ng/cm^2.day)	2.715	1.802	1.360	0.811	0.608	0.519	0.590	0.448	0.314	0.273	0.179

Data presented are mean values of triplicate preparation.

Average Device Wt = 135.8 g Average Surface Area of 125cc Device = 150.9 cm^2

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Table III
Solubility of Potential Gel Migrant
(at room temperature)

	in Water		in Porcine Serum
Compound/Analyst	A*	B**	A
	ppm		
D3	1.306	1.500	0.292
D4	0.020	0.050	0.502
D5	0.042	0.010	0.211
D6	0.136	~0	0.556
D9	0.362	NA	0.549
Pt	—	—	7.298

NA = Not Applicable, at least one of the replicates had a ND value.

*A - Analysis Performed by Mentor

**B - Data Recorded by Dow Corning

Table IV

The Release of LMW Siloxanes and Pt from Gel Filler of Gel-Filled Mammary Implants into Porcine Serum

Compound	Total Available Migrant[1,2]	Total Migrant in Saturated Porcine Serum	Total Allowable Migrant after Consideration for Solubility in Serum	Maximum Migrant Release during 120-day Immersion
	A	B	C	
	ug			
D3	ND	65.70	ND	NA
D4	73.33	112.95	73.33	0.04
D5	335.43	47.48	47.48	2.84
D6	664.06	125.10	125.10	1.39
D9	1139.36	123.53	123.53	NA
Pt	679.00	1642.07	528.52	4.06

Note: All estimates are for 125cc device (Gel Filler Wt: 135.8 g) in 225 mL porcine serum

[1] Report CP357 - Methylene Chloride Extractables Analysis of Gel Filler

[2] Manufacturer's information, gel filler contains ~5 ppm Pt. Total Pt Analysis of Gel Filler

C equals the lower value of A and B

Figure 1 Accumulated Release of Pt from Gel-Filled Implant into Porcine Serum